Synopsis

Liposomes were first described 20 years ago. Only recently have they been extensively studied for their use as carriers in the delivery of drugs, antigens, hormones, enzymes and other biologicals. They are the wave of the future.

Liposomes: a new generation of drug and vaccine carriers

[1]Pang N. Shek, PhD; [2]Raymond F. Barber, PhD

[1]Head, Immunology Group, Defence and Civil Institute of Environmental Medicine, Downsview; Assistant Professor of Clinical Biochemistry, University of Toronto; [2]Research Fellow, Defence and Civil Institute of Environmental Medicine, Downsview, Ont.

Liposomes are phospholipid vesicles which were first described[1] two decades ago; they have since been widely used as models of biologic membranes. In recent years, liposomes have been extensively studied for their potential to serve as carriers for the delivery of drugs, antigens, hormones, enzymes, and other biologicals. Because liposomes are composed of naturally-occurring substances, they have the distinct advantages of being nontoxic and biodegradable. Some examples of human diseases where lipsome-incorporated agents have been used or may likely be used in the future for clinical treatment are listed in Table I.

Biologically-active materials encapsulated within liposomes are protected to various extents from immediate dilution or degradation in vivo. This protective property promotes the delivery of entrapped drugs to the target organ by preventing a premature drug release after its administration to the body. For example, the encapsulation of a highly toxic antimony-containing drug in liposomes has been shown to be useful in reducing otherwise fatal systemic side effects in the treatment of experimental leishmaniasis.[2] Major improvements in the therapeutic index of amphotericin B encapsulated in liposomes have been demonstrated in counteracting systemic fungal infections among cancer patients.[3] The liposomal entrapment of this antifungal drug causes a remarkable reduction in toxicity. Liposomes have also been found effective for the delivery of labile macromolecules, like insulin[4] and factor VIII[5] via the oral route. The liposomal envelope again provides some protection of the entrapped materials, in this case from gastrointestinal degradation. The entrapment of weakly immunogenic protein antigens in liposomes has been shown to potentiate the specific immune response to the entrapped antigen.[7] Furthermore, liposome-mediated immune enhancement also substantially reduces or eliminates irritable hypersensitivity reactions at the site of injection.[8]

In this review, we will summarize the main biochemical and physical features of liposomes and highlight the potential application of liposomes in drug targeting and immunopotentiation.

Physicochemical properties of liposomes

Many membrane lipids will spontaneously form bimolecular lamellae, or bilayers, when hydrated in aqueous solution. These bilayers then round up to form vesicles, and in the

Reprinted from MODERN MEDICINE April 1986

Fig. 1. — Liposome structure. The multiple concentric lipid bilayers of a multilamellar liposome are clearly indicated by the freeze-fracture electron micrograph illustrated in this figure. The arrangement of the amphipathic phospholipid molecules is shown in the schematic. The polar head-groups situate themselves on the bilayer surface with the nonpolar fatty acid side-chains extending into the bilayer core. Cholesterol molecules align themselves parallel to the phospholipid molecules with the hydroxyl moiety in the headgroup region. The rigid sterol nucleus intercalates in between the acyl chains and reduces their mobility in fluid bilayers. Other amphipathic and nonpolar agents penetrate the lipid matrix to an extent determined by their inherent hydrophobicity. Water-soluble agents can be entrapped in the aqueous compartment bounded by polar headgroups of the lipid molecules.

process entrap some of the hydrating solution. Liposomes are formed from amphipathic lipid molecules, with phospholipids being the major component. Most commonly, phosphatidylcholine is used as the primary constituent. Other lipids such as phosphatidylethanolamine, phosphatidylserine, sphingomyelin, glycolipids, and sterols are also frequently added.

Biologically-active substances can be incorporated either within the bilayers themselves or be trapped within the internal aqueous compartments. Hydrophobic and amphipathic drugs such as amphotericin B[9] and indoxole[10] can be combined with the lipids prior to liposome formation. Because of their hydrophobicity, these compounds will intercalate into the lipid bilayer. Hydrophilic drugs such as fluorodeoxyuridine[11] and cytosine arabinoside[12] are added during liposome formation and remain in the aqueous phase. Figure 1 shows the interior view of a multilamellar liposome, obtained by freeze-fracture electron microscopy; a schematic representation of the membrane structure is also illustrated.

Liposome preparation

Liposomes can be prepared by a growing number of methods[13] which produce vesicles differing in size, number of lamellae, and the efficiency with which water-soluble compounds are trapped (Fig. 2). The method first described for making liposomes simply involved hydrating a thin film of dried lipids.[1] This still commonly-used method produces multilamellar vesicles (MLV). Each vesicle may be visualized as an onion-like structure with many concentric bilayers enclosing an equal number of aqueous compartments (Fig. 1).

Synopsis

Liposomes work by protecting various encapsulated, biologically-active materials from immediate dilution or degradation in vivo. This promotes the delivery of entrapped drugs to the target organ by preventing premature release.

Large unilamellar vesicles (LUV) can be produced by different methods[13-15] and vesicles as large as 100 μm have been reported.[10] Small unilamellar vesicles (SUV) can be prepared simply by sonicating the large multilamellar vesicles.

Entrapment of water-soluble drugs

The amount of polar drug entrapped depends on both its water solubility and the volume entrapped by the particular vesicle. While SUV have the smallest internal volume to lipid ratio, LUV possess a large internal aqueous space and trap solutes most efficiently.[9] Entrapment efficiencies approaching 100% have been reported for various antineoplastic agents, when an induced membrane potential is used to load the vesicles.[21] Alternatively, some water-soluble drugs have been modified by the addition of hydrophobic anchors (e.g., palmitate) to improve entrapment.[22-24]

Release of entrapped drugs

The release of liposome-entrapped agents is related to the stability of the lipid membranes. The permeability of lipid bilayers is controlled by headgroup interactions,[24] and more importantly by the tightness with which the fatty acid side-chains pack together. The permeability of liposomes prepared from various phosphatidylcholines increases with increasing temperature, decreasing chain length, and increasing unsaturation.[25]

Individual phospholipids undergo a phase transition at characteristic temperatures (e.g., 41 C for dipalmitoylphosphatidylcholine). The transition is from a gel phase, characterized by restricted molecular motion, to a liquid-crystalline phase characterized by a high degree of molecular motion, and therefore increased permeability and solute release. During this phase transition, the permeability of many solutes displays a maximum as a result of transient defects which facilitate transmembrane movement.

Table I — Examples of liposome-mediated delivery of drugs and biologic agents for the treatment of human diseases

Human diseases	Liposome-incorporated agents
Attempted human trials:	
Cancer	Methotrexate[6]
Diabetes mellitus	Insulin[4]
Enzyme storage disease	Glucocerebroside: β-glucosidase[14]
Hemophilia A	Factor VIII[5]
Rheumatoid arthritis	Cortisol palmitate[15]
Respiratory distress syndrome	Surfactant[16]
Systemic fungal infection	Amphotericin B[3]
Future prospectives suggested by animal studies:	
Cutaneous diseases	Triamcinolone[61]
Demyelinating diseases	Myelin basic protein[17]
Gout	Modified uricase[79]
Metastatic tumors	Macrophage activating factors[42]
Leishmaniasis	Antimonials[2]
Ophthalmic diseases	Penicillin G[10]

The increase in permeability which is observed in the region of the phase transition has been used to improve drug delivery. For example, the delivery of methotrexate to locally heated tumors in vivo can be enhanced by using liposomes with a transition temperature slightly above the physiologic range.[26] Heat sensitive and antibody-targeted liposomes have recently been used successfully in vitro.[7]

Cholesterol can be used to modulate bilayer permeability by virtue of its ability to effect side-chain packing.[25] Incorporation of cholesterol in gel-phase bilayers increases permeability while incorporation in liquid-crystalline

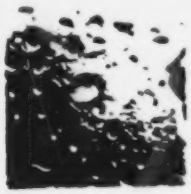
	Multilamellar Vesicles (MLV)	Large Unilamellar Vesicles (LUV)	Small Unilamellar Vesicles (SUV)
			
Diameter (nm)	400-10.000	60-10.000	20-64
Typical Entrapment Efficiencies	5-15%	35-65%	0.5-1.0%

Fig. 2 — Types of Liposomes. Representative freeze-fracture electron micrographs of the three major types of lipid vesicles are shown; the range of diameters and entrapment efficiencies as reported in the literature[18,20,53,78] are indicated. The entrapment efficiencies noted are for water-soluble compounds passively trapped during vesicle formation.

bilayers reduces permeability.[77]

In essence, the modulation of liposome permeability and the release of entrapped solutes are dependent, at least in part, on the nature of the phospholipids used and the amount of cholesterol incorporated.

Liposome-mediated drug delivery

Natural targeting. The amount of drug necessary to achieve a therapeutic effect in combating many diseases usually requires systemic levels which are cytotoxic to normal cells. The prospect of successfully targeting drugs to a specific site, thereby reducing the effective dose required, is very attractive. The advantages of liposome-mediated drug targeting are perhaps most evident when one considers cancer chemotherapy; a recent review of this approach has been provided by Poznansky and Juliano.[31]

While considerable success has been achieved in vitro, the in vivo application of liposomes for drug delivery is confounded by the impenetrability of the continuous vascular endothelium found in most tissues.[9] Furthermore, intravenously-administered liposomes are predominantly taken up by phagocytes. The greatest successes with systemically delivered liposomal preparations have, for the above-mentioned reasons, been achieved when the desired targets are confined within the intravascular space or the reticuloendothelial system. Several examples of liposome-mediated drug delivery are discussed in the following sections, including anti-infective agents, macrophage activating factors, and antineoplastic agents.

Enhancement of the therapeutic efficacy of antibiotics. Administration of the anthracycline antibiotic, doxorubicin (adriamycin), encapsulated within liposomes, has been shown by many groups to improve the therapeutic index of this cardiotoxic antitumor drug in animal models.[41] A further enhancement of antitumor activity and reduced cardiotoxicity resulting in a significant extension of lifespan has been reported in leukemic mice using cardiolipin-containing liposomes.[42] Success has also been reported in the treatment of a murine liver tumor using liposomally entrapped doxorubicin.[43] Perhaps not surprisingly, liposomal encapsulation reduces the toxicity of violamycin, another anthracycline.[44]

The mechanism by which liposome encapsulation improves the therapeutic effectiveness of anthracycline antibiotics is not at all clear, but appears to involve altered pharmacokinetics, metabolism, and immune responses. The benefits of reduced toxicity are significant.

A number of anti-infective agents are also quite toxic and liposomal delivery has been shown to lessen systemic toxicity as well. Considerable success has been achieved in the treatment of systemic mycoses, including cryptococcus,[45] histoplasmosis,[46] and candidiasis as discussed later.

Amphotericin B is a highly toxic lipophilic polyene antibiotic that interacts with ergosterol in fungal cell membranes. Unfortunately, this very potent antifungal agent also combines

Synopsis

There are many methods of producing liposomes. The first method (one still commonly used) involves hydrating a thin film of dried lipids. This produces multilamellar vesicles. Their application is on the horizon

with cholesterol in animal cell membranes. The toxicity of this drug has been very markedly reduced by its incorporation into liposomes without any loss of fungicidal potency.[63] Encapsulation allows for the safe administration of otherwise fatal doses of amphotericin in mice with disseminated candidiasis. Furthermore, a single high dose of the liposome-encapsulated drug can cure severely infected and profoundly neutropenic mice which are completely unresponsive to therapy with the free drug.[65] The improved therapeutic effect of liposomally entrapped amphotericin B appears to be a result of a reduced propensity for amphotericin B to intercalate into mammalian cell membranes.[41] Limited clinical trials with human cancer patients have begun and appear very promising.[4]

Targeting of liposome-encapsulated drugs to the RES. The homing of systemically-injected liposomes to the reticuloendothelial system (RES) has been exploited for passive delivery. The treatment of leishmaniasis, for example, has been particularly successful because the parasite inhabits macrophages. The passive targeting achieved by encapsulating antimonials within liposomes decreases the effective dose so significantly that, even though the entrapped drug is more toxic than the free drug, there is a 30- to 40-fold increase in therapeutic index.[59] The drug-containing liposomes are taken up by infected macrophages and fuse with vacuoles containing the parasites, apparently without any of the drug entering the cell cytoplasm.[59]

Liposomal encapsulation potentiates the activity of a number of therapeutic agents in counteracting intraphagocytic bacterial and viral infections (Table II). As an example, liposomal encapsulation of ampicillin enhances its antimicrobial efficacy by at least 80-fold in mice infected with Listeria monocytogenes.[40]

Table II — List of organisms infecting phagocytic cells of the RES more susceptible to liposomally delivered drugs

Organism	Drug
Brucella abortus	Gentamycin[66]
Listeria monocytogenes	Ampicillin[40]
Staphylococcus aureus	Aminoglycosides[67] Dihydrostreptomycin[68]
Escherichia coli	Streptomycin, Chloramphenicol[69]
Salmonella typhimurium	Cephalothin[70]
Mycobacterium tuberculosis	Streptomycin[71]
Rift Valley fever virus	Ribavirin[72]

Activation of the tumoricidal capacity of macrophages. The natural targeting of liposomes to phagocytes allows for the possibility of activating host macrophages to become cytotoxic to neoplastic or virus-infected cells as an adjunct to other therapies. Considerable experimentation has demonstrated the enhanced ability of various immunomodulatory agents to stimulate macrophages when encapsulated in liposomes.[41] The potential for liposome-mediated macrophage activation, and also the delivery of specific antiviral agents, has recently been reviewed by Koff and Fidler.[42]

Liposome-entrapped muramyl dipeptide and, more promisingly, various lipophilic analogues, have been used to activate macrophages to become tumoricidal both in vitro and in vivo.[43,44] Destruction of murine pulmonary and lymph node melanoma metastases has been successfully promoted in vivo by the systemic delivery of these preparations.[45,46] The eradication of pulmonary tumors is apparently the result of the migration of circulating monocytes into alveolar tissues following the phagocytosis of liposomes.[45]

Synopsis

Improved opacification of the liver and spleen on computed tomographic images has been effected in animals by IV administration of radiographic contrast agents when entrapped within liposomes.

Antibody-mediated targeting. The attractiveness in the use of liposomes as drug carriers is related to their potential in the targeting of encapsulated drugs to *specific* sites of the body. In principle, this may be achieved by the incorporation of specific ligands, e.g., antibodies, which can recognize and interact with surface antigens or other markers of the target cell. A schematic representation of antibody-mediated targeting of a liposome-encapsulated drug for the selective destruction of the target cell is shown in Fig. 3.

Different methods for the covalent linkage of immunoglobulins to the liposome surface have been developed.[47-49] So far, the application of the concept of antibody-mediated liposome targeting has been most successful in in vitro studies. Lipid vesicles conjugated with antibodies directed against target cell antigens have been shown to interact preferentially with the target.[50] Furthermore, the loaded drug is delivered more effectively to the target cell than to unrelated cells.[51,52] The efficiency of delivery is related to the physicochemical characteristics, e.g., size, lipid composition and surface charge of the liposomes employed as well as the specificity of the surface-coated antibodies.[53]

The delivery of liposome-encapsulated drugs to specific sites in vivo, however, is impeded by at least two apparent obstacles. The first obstacle is the preferential clearance of liposomes by the reticuloendothelial system. This may be overcome by presaturating the phagocytic cells with a large dose of empty liposomes prior to the administration of antibody-coated and drug-laden vesicles. The second obstacle is the difficulty of intravenously injected liposomes to escape from the intravascular compartment. This restriction, however, does not apply to sinusoidal capillaries where the discontinuous nature of the endothelium allows the liposomes to pass through more freely.[54] The endothelial barrier can also be bypassed by the selection of other routes of administration, e.g., by intraperitoneal, intralymphatic, and topical applications. As a matter of fact, the intraperitoneal administration of actinomycin D-loaded liposomes, bearing surface-conjugated antitumor IgM antibodies, is preferentially effective in the treatment of peritoneal tumors.[55] The potent and selective in vivo antitumor activity is attributable to the specific interactions between antibody-conjugated liposomes and the tumor targets.

Other therapeutic and diagnostic applications. Liposomes may also be used for diagnostic applications. For example, improved opacification of the liver and spleen on computed tomographic images has been effected in animals by the intravenous administration of various radiographic contrast agents entrapped within liposomes.[56,57] In humans, technetium-99m-labelled liposomes have been used to visualize certain malignancies[80] and focal inflammatory lesions[81] by scintigraphy.

Recent evidence suggests that circulating vesicles with a prolonged half-life (16 to 20 hours) are cleared to a considerable extent by bone marrow cells.[58] Thus, the possibility of passively targeting drug-containing liposomes to these cells should also be considered for the chemotherapy of bone marrow-related malignancies.

Liposomes have proven useful as controlled delivery devices in extravascular regions when applied directly to the site of required action. Topical ophthalmic application of liposomal preparations enhances the absorption of a number of antibiotics, incuding penicillin G[10] and pimaricin.[59] The use of liposomes for topical cutaneous application remains an area for exploitation. An appropriately prepared drug-containing liposomal formulation should enable a prolonged and sustained release of

Synopsis

The effective targeting of liposomes to a selective site within the body depends on several factors; these include the biochemical composition, physical size, and the surface-associated ligands of liposomes used.

the drug to the skin at the site of application. Improved cutaneous absorption of methotrexate[40] and triamcinolone[41] has been documented using this strategy.

Potential as a vaccine carrier

Advantageous properties. The biodegradable and relatively nonimmunogenic nature of most liposomes render them ideal candidates to be used as vaccine carriers. When the liposome was first used as an immunologic carrier, it was expected to reduce or eliminate the immune response because the entrapped antigen was supposedly shielded from the host's immune system. Contrary to this initial expectation, liposome encapsulation was found to substantially potentiate the antibody response of the immunized host to the entrapped antigen.[42] Unlike other potent immunoadjuvants, e.g., complete Freund's adjuvant, which are unacceptable for human use because of irritable necrotic reactions at the site of injection, the injection of antigen-containing liposomes does not induce local granuloma formation.[43] The entrapped antigen also does not elicit cutaneous delayed hypersensitivity reactions after secondary immunizations.[5] The elimination of this irritable side effect is another advantage of using liposomes as a vaccine carrier.

Liposome-mediated immunopotentiation. Liposomes have been used as carriers for model antigens and vaccines in many studies. Various kinds of antigenic substances, including proteins, carbohydrates, lipids, and glycolipids, have been employed for incorporation. A list of some of the studies using protein antigens for encapsulation is shown in Table III. In most if not all of the studies, the association of a protein antigen with a lipid vesicle by internal entrapment or surface linkage renders the antigen much more immunogenic. This immunopotentiating effect applies to both humoral and cell-mediated immune responses. The adjuvanticity of liposomes has been exploited more recently to promote the immunogenicity of synthetic peptide antigens. For example, the covalent linkage to liposomes of a synthetic peptide corresponding to a short amino acid sequence of hepatitis B surface antigen (HBsAg) can induce significant levels of anti-HBsAg antibodies in the immunized host.[44] The implication of this study is far reaching in terms of circumventing the need to purify viral antigens from the sera of hepatitis carriers.

Table III — Examples of protein antigens incorporated in liposomes for immunopotentiation

Protein antigens	Liposome composition*
Adenovirus antigen	10DPPC:7.5CSL:1PA[73]
Bovine serum albumin	7PC:2CSL:1PA[74,75]
Diphtheria toxoid	7PC:2CSL:1DCP[62]
Gross-virus surface antigen	7PC:2CSL:1DCP[76]
Hepatitis B surface antigen	5PC:5CSL:1DCP[77]

**The constituting lipids are indicated in molar ratios. CSL = cholesterol; DCP = dicetylphosphate; DPPC = dipalmitoylphosphatidylcholine; PA = phosphatidic acid; PC = phosphatidylcholine.*

Specific immunomodulation. Liposome formulations can be designed to serve as a heterobifunctional carrier. The liposomal membrane provides a surface for the covalent attachment of antigens and the liposomal interior allows for the entrapment of soluble mediators. This concept has been advanced in the engineering of a liposomal vesicle containing surface-conjugated protein antigens and an internally encapsulated drug.[45] The use of this type of loaded vesicles is found to be effective in regulating the specific immune response to

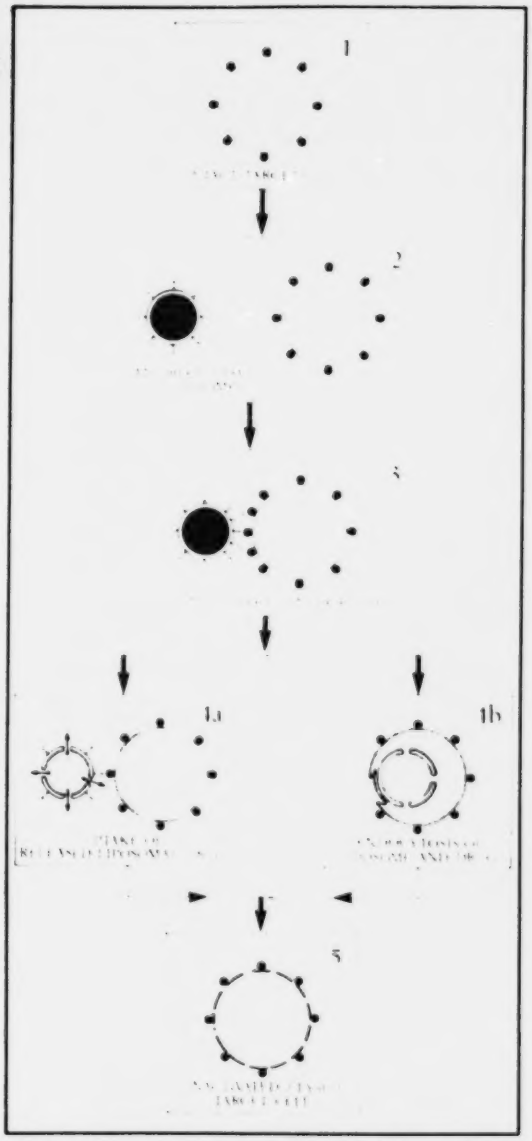


Fig. 3 — Schematic illustration of target cell destruction by antibody-coated and drug-containing liposomes. 1) An intact target cell, e.g., a tumor cell, bearing cell-surface associated antigens. 2) Target cell approached by a drug-loaded liposome coated with target-specific antibodies. 3) Interaction between the liposome and the target cell via antibody-mediated recognition and binding. 4) Delivery of liposomal drug either by a) the uptake of drug leaked from the liposome in the vicinity of the target, or b) the ingestion of the liposome-drug complex by the target cell. 5) Drug-mediated inactivation/lysis of the target cell.

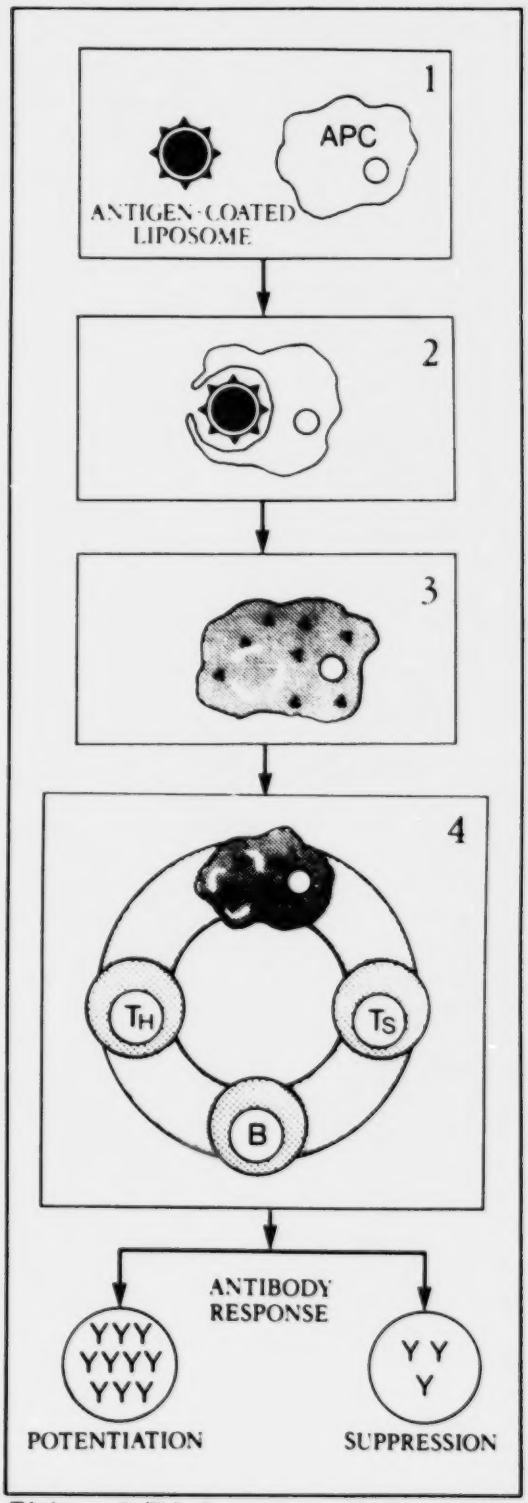


Fig. 4 — Liposome-mediated modulation of the antibody response. 1) A liposome containing surface-conjugated antigen and encapsulated immunomodulating agent approached by an antigen processing/presenting cell (APC), e.g., a macrophage. 2) Endocytosis of the liposome complex by the APC. 3) Degradation and processing of the internalized antigens. 4) Presentation of processed antigens to appropriate interacting lymphoid cells, e.g., T-helper cell (T_H), T suppressor cell (T_S), and bone marrow-derived cell (B), the interaction being influenced by the release of modulating agent from the APC. The net effect of this liposome-mediated and antigen-directed targeting of an immunomodulator may result in the potentiation or suppression of the specific antibody response.

the liposome-attached antigen. The engendered antibody response can be enhanced or suppressed depending on the dose of the encapsulated drug administered. A comparable regulation of the immune response, however, was not observed by the administration of a similar dose of *free* drug in control studies. Therefore, the specific immunomodulation mediated by liposome-encapsulated drugs may be due to antigen-directed targeting of the drug effect to the interacting lymphoid cells (Fig. 4). The antigen-processing cell (APC), having incorporated the antigen-coated and drug-loaded liposomes, serves to deliver the drug more directly to the interacting cells, namely bone-marrow derived (B), thymus-derived helper (T_H) and suppressor (T_S) cells. The selective action of the drug on each cell population may in turn influence the resulting magnitude of the antibody response. This approach of simultaneously delivering an antigen and a regulatory drug suggests a potentially useful clinical application of liposomes as effective immunomodulating agents.

Effective carrier for subunit vaccines. The conventional method of subunit vaccine production in general requires the extraction and purification of the antigenic moieties derived from whole viral or bacterial organisms. The vaccine preparation procedures are usually cumbersome, time-consuming, and expensive. Despite a high standard of purification procedures, the presence of trace contaminating substances may still cause some common irritable, and occasionally devastating, side effects among susceptible individuals. With the advance of new biochemical techniques, specifically recombinant DNA technology, well-defined portions corresponding to the antigenic determinants of macromolecules can be readily produced. Because small antigen fragments are usually not very immunogenic, the requirement of a suitable immunogenic carrier is necessary. The successful exploitation of the liposome as a vaccine carrier for well-defined antigen fragments may revolutionize the efficacy and safety of future vaccines.

References

1. BANGHAM AD, STANDISH MM, WATKINS JC: Diffusion of univalent ions across the lamellae of swollen phospholids. *J Mol Biol 1965; 13:238-252*
2. ALVING CR, STECK EA, CHAPMAN WL Jr, WAITS VB, HENDRICKS LD, SWARTZ GM, HANSON WL: Therapy of leishmaniasis: superior efficacies of liposome-encapsulated drugs. *Proc Natl Acad Sci (USA) 1978; 75:2959-2963*
3. LOPEZ-BERESTEIN G, FAINSTEIN V, HOPFER R, MEHTA K, SULLIVAN MP, KEATING M, ROSENBLUM MG, MEHTA R, LUNA M, HERSH EM, REUBEN J, JULIANO RL, BODEY GP: Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study. *J Infect Dis 1985; 151:704-710*
4. PATEL HM, HARDING NG, LOGUE F, KESSOR C, MacCUISH AC, MACKENZIE JC, RYMAN BE, SCOBIE I: Intrajejunal absorption of liposomally entrapped insulin in normal man. *Biochem Soc Trans 1978; 6:784-785*
5. HEMKER HC, MULLER AD, HERMENS WH, ZWAAL RFA: Oral treatment of haemophilia A by gastrointestinal absorption of factor VIII entrapped in liposomes. *Lancet 1980; 1:70-71*
6. TACHIBANA M, DEGUCHI N, HAGIWARA M, MURAI M, HATA M, TAZAKI H: Experimental and clinical study of liposome entrapped anticancer drugs. *J Japan Soc Cancer Therapy 1982; 17:578*
7. SHEK PN: Appications of liposomes in immunopotentiation. In: *Immunotoxicity*. MULLEN PW (ed.) Springer Verlag, Heidelberg, 1984, pp. 103-125
8. GREGORIADIS G, ALLISON AC: Entrapment of proteins in liposomes prevents allergic reactions. *FEBS Lett 1974; 45:71-74*
9. LOPEZ BERESTEIN G, MEHTA R, HOPFER RL, MILLS K, KASI L, MEHTA K, FAINSTEIN V, LUNA M, HERSH EM, JULIANO R: Treatment and prophylaxis of disseminated infection due to Candida albicans in mice with liposome encapsulated amphotericin B. *J Infect Dis 1983; 147:939-945*
10. SCHAEFFER HE, KROHN DL: Liposomes in topical drug delivery. *Invest Ophthalmol Vis Sci 1982; 22:220-227*
11. JULIANO R, LOPEZ BERESTEIN G, MEHTA R, HOPFER R, MEHTA K, KASI L: Pharmacokinetic and therapeutic consequences of liposomal drug delivery: fluorodeoxyuridine and amphotericin B as examples. *Biol Cell 1983; 47:39-46*
12. TSUKADA K, UEDA S, OKADA R: Preparation of liposome encapsulated anti tumor drugs: relationships between lipophilicity of drugs and in vitro drug release. *Chem Pharm Bull 1984; 32:1929-1935*
13. SZOKA F Jr, PAPAHADJOPOULOS D: Comparative properties and methods of preparation of lipid vesicles (liposomes). *Ann Rev Biophys Bioeng 1980; 9:467-508*
14. GREGORIADIS G: Liposomes in therapeutic and preventive medicine: the development of the drug carrier concept. In: *Liposomes and Their Uses in Biology and Medicine*. PAPAHADJOPOULOS D (ed.) The New York Academy of Sciences, New York, NY, 1978, pp. 343-365
15. DeSILVA M, HAZELMAN BL, PAGE THOMAS DP, WRAIGHT P: Liposomes in arthritis: a new approach. *Lancet 1979; 1:1320-1322*
16. IVEY H, ROTH S, KATTWINKEL J: Nebulization of sonicated phospholipids (PL) for treatment of respiratory distress syndrome (RDS) of infancy. *Pediatr Res 1977; 11:573*
17. STREJAN GH, GILBERT JJ, ST LOUIS J: Suppression of chronic relapsing experimental allergic encephalomyeletis in strain 13 Guinea pigs by administration of liposome-associated myelin basic protein. *J Neuroimmunol 1984; 7:27-41*
18. HOPE MJ, BALLY MB, WEBB G, CULLIS PR: Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochim Biophys Acta 1985; 812:55-65*
19. OKU N, SCHEERE JF, MACDONALD RC: Preparation of giant liposomes. *Biochim Biophys Acta 1982; 692:384-388*
20. POZNANSKY MF, JULIANO RL: Biological approaches to the controlled delivery of drugs: a critical review. *Pharmacol Rev 1984; 36:277-336*
21. MAYER LD, BALLY MB, HOPE MJ, CULLIS PR: Uptake of antineoplastic agents into large unilamellar vesicles in response to a membrane potential. *Biochim Biophys Acta 1985; 816:294-302*
22. SHAW JH, KNIGHT CG, DINGLE JT: Liposomal retention of a modified anti-inflammatory steroid. *Biochem J 1976; 158:473-476*
23. GOUNDALKAR A, MEZEI M: Chemical modification of triamcinolone acetonide to improve liposomal encapsulation. *J Pharm Sci 1984; 73:834-835*
24. SINGER M: Permeability of phosphatidylcholine and phosphatidylethanolamine bilayers. *Chem Phys Lipids 1981; 28:253-267*
25. DEMEL RA, GEURTS van KESSEL WSM, van DEENEN LLM: The properties of polyunsaturated lecithins in monolayers and liposomes and the interactions of these lecithins with cholesterol. *Biochim Biophys Acta 1972; 266:26-40*
26. WEINSTEIN JN, MAGIN RL, YATVIN MB, ZAHARKO DS: Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. *Science 1979; 204:188-191*
27. SULLIVAN SM, HUANG L: Preparations and characterization of heat-sensitive immunoliposomes. *Biochim Biophys Acta 1985; 812:116-126*
28. BLOCK KE: Sterol structure and membrane function. In: *Critical Reviews in Biochemistry, Volume 14*. FASMAN GD (ed.) CRC Press, Boca Raton, 1983, pp. 47-92
29. BITTMAN R, BLAU L: The phospholipid-cholesterol interaction. Kinetics of water permeability in liposomes. *Biochemistry 1972; 11:4831-4839*

30. POSTE G, BUCANA C, RAZ A, BUGELSKI P, KIRSH R, FIDLER IJ: Analysis of the fate of the systemically administered liposomes and implications for their use in drug delivery. *Cancer Res 1982; 42:1412-1422*
31. JULIANO RL, LOPEZ BERESTEIN G: New lives for old drugs. Liposomal drug delivery systems reduce the toxicity but not the potency of certain chemotherapeutic agents. *Pharm International 1985; 6:164-167*
32. RAHMAN A, WHITE G, MORE N, SCHEIN PS: Pharmacological, toxicological, and therapeutic evaluations in mice of doxorubicin entrapped in cardiolipin liposomes. *Cancer Res 1985; 45:796-803*
33. MAYHEW E, RUSTUM Y, VAIL WJ: Inhibition of liver metastases of M5076 tumor by liposome-entrapped adriamycin. *Cancer Drug Delivery 1983; 1:43-58*
34. HANSCHMANN H, HARTL A, HOFFMAN H, LOBER G: Reduction of the acute toxicity of the anthracycline antibiotic violamycin BI in mice and rats by liposomal encapsulation. *Studia Biophysica 1984; 104:227-232*
35. GRAYBILL JR, CRAVEN PC, TAYLOR RL, WILLIAMS DM, MAGEE WE: Treatment of murine cryptococcosis with liposome associated amphotericin B. *J Infect Dis 1982; 145:748-752*
36. TAYLOR RL, WILLIAMS DM, CRAVEN PC, GRAYBILL JR, DRUTZ DJ, MAGEE WE: Amphotericin B in liposomes: a novel therapy for histoplasmosis. *Ann Rev Respir Dis 1982; 125:610-611*
37. NEW RRC, CHANCE ML, HEATH S: Antileishmanial activity of amphotericin and other antifungal agents entrapped in liposomes. *J Antimicrobial Chemother 1981; 8:371-381*
38. LOPEZ-BERESTEIN G, HOPFER RL, MEHTA R, MEHTA K, HERSH EM, JULIANO RL: Liposome-encapsulated amphotericin B for treatment of disseminated Candidiasis in neutropenic mice. *J Infect Dis 1984; 150:278-283*
39. HEATH S, CHANCE ML, NEW RRC: Quantitative and ultrastructural studies on the uptake of drug loaded liposomes by mononuclear phagocytes infected with *Leishmania donavani*. *Molec Biochem Parasitology 1984; 12:49-60*
40. BAKKER-WOUDENBERG IAJM, LOKERSE AF, ROERDINK FH, REGTS D, MICHEL MF: Free versus liposome-entrapped ampicillin in treatment of infection due to *Listeria monocytogenes* in normal and athymic (nude) mice. *J Infect Dis 1985; 151:917-924*
41. POSTE G, KIRSH R: Liposome-encapsulated macrophage activation agents and active non-specific immunotherapy of neoplastic disease. In: *Cell Function and Differentiation, Part A*. Alan R. Liss, Inc., New York, NY, 1982. pp. 309-319
42. KOFF WC, FIDLER IJ: The potential use of liposome-mediated antiviral therapy. *Antiviral Res 1985; 5:179-190*
43. LOPEZ-BERESTEIN G, MEHTA K, MEHTA R, JULIANO RL, HERSH EM: The activation of human monocytes by liposome-encapsulated muramyl dipeptide analogues. *J Immunol 1983; 130:1500-1502*
44. SCHROIT AJ, GALLIGIONI E, FIDLER IJ: Factors influencing the *in situ* activation of macrophages by liposomes containing muramyl dipeptide. *Biol Cell 1983; 47:87-94*
45. FIDLER IJ, SCHROIT AJ: Synergism between lymphokines and muramyl dipeptide encapsulated in liposomes: *in situ* activation of macrophages and therapy of spontaneous cancer metastases. *J Immunol 1984; 133:515-518*
46. PHILLIPS NC, MORAS ML, CHEDID L, LEFRANCIER P, BERNARD JM: Activation of alveolar macrophage tumoricidal activity and eradication of experimental metastases by freeze-dried liposomes containing a new lipophilic muramyl dipeptide derivative. *Cancer Res 1985; 45:128-134*
47. LESERMAN LD, BARBET J, KOURILSKY FM, WEINSTEIN JN: Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. *Nature 1980; 288:602-604*
48. HEATH TD, MACHER BA, PAPAHADJOPOULOS D: Covalent attachment of immunoglobulins to liposomes via glycosphingolipids. *Biochim Biophys Acta 1981; 640:66-81*
49. MARTIN FJ, PAPAHADJOPOULOS D: Irreversible coupling of immunoglobulin fragments to preformed vesicles. *J Biol Chem 1982; 257:286-288*
50. HEATH TD, FRALEY RT, PAPAHADJOPOULOS D: Antibody targeting of liposomes: Cell specificity obtained by conjugation of F(ab')2 to vesicle surfaces. *Science 1981; 210:539-541*
51. LESERMAN LD, MACHY P, BARBET J: Cell-specific drug transfer from liposomes bearing monoclonal antibodies. *Nature 1981; 293:226-228*
52. HEATH TD, MONTGOMERY JA, PIPER JR, PAPAHADJOPOULOS D: Antibody targeted liposomes: Increase in specific toxicity of methotrexate-gamma-aspartate. *Proc Natl Acad Sci (USA) 1983; 80:1377-1381*
53. WEINSTEIN JN, LESERMAN LD: Liposomes as drug carriers in cancer chemotherapy. *Pharmacol Ther 1984; 24:207-233*
54. POSTE G: Drug targeting in cancer therapy. In: *Receptor-Mediated Targeting of Drugs*. GREGORIADIS G, POSTE G, SENIOR J, TROUET A (eds.). Plenum Press, New York, NY, 1985. pp. 427-474
55. HASHIMOTO Y, SUGAWARA M, MASUKO T, HOJO H: Antitumor effect of actinomycin D entrapped in liposomes bearing subunits of tumor-specific monoclonal immunoglobulin M antibody. *Cancer Res 1983; 43:5328-5334*
56. RYAN PJ, DAVIS MA, deGAETA LR, WODA B, MELCHIOR D: Liposomes loaded with contrast material for image enhancement in computed tomography. *Radiology 1984; 152:759-762*
57. SELTZER SE, SHULKIN PM, ADAMS DF, DAVIS MA, HOEY GB, HOPKINS RM, BOSWORTH ME: Usefulness of liposomes carrying iosefamate for CT opacification of liver and spleen. *Am J Roentgen 1984; 143:575-579*
58. SENIOR J, CRAWLEY JCW, GREGORIADIS G: Tissue distribution of liposomes exhibiting long half-lives in the circulation after intravenous injection. *Biochim Biophys Acta 1985; 839:1-8*
59. BELMONT J, SMOLIN G, CONDON D: Pimaricin: Corneal penetration via a liposome vehicle. *Invest Ophthal Vis Sci 1981; 20:109*
60. PATEL HM: Liposomes as a controlled-release system. *Biochem Soc Trans 1985; 13:513-516*
61. MEZEI M, GULASEKHAREM V: Liposomes: A selective drug delivery system for the topical route of administration: gel dosage form. *J Pharm Pharmacol 1982; 34:473-474*
62. ALLISON AC, GREGORIADIS G: Liposomes as immunological adjuvants. *Nature (London) 1974; 252:252*
63. ALLISON AC, GREGORIADIS G: Liposomes as immunological adjuvants. In: *Recent Results in Cancer Research*. MATHE G, FLORENTIN I, SIMMLER MC (eds.) Springer Verlag, Heidelberg, 1976. pp. 58-64
64. NEURATH AR, KENT SBH, STRICK N: Antibodies to hepatitis B surface antigen (HBsAg) elicited by immunization with a synthetic peptide covalently linked to liposomes. *J Gen Virol 1984; 65:1009-1014*
65. SHEK PN, LOPEZ NG, HEATH TD: Immune response mediated by liposome associated protein antigens. IV. Modulation of antibody formation by vesicle encapsulated methotrexate. *Immunology 1986; 57:153-157*
66. DEES C, FOUNTAIN MW, TAYLOR JR, SCHULTZ RD: Enhanced intraphagocytic killing of *Brucella abortus* in bovine mononuclear cells by liposome-containing gentamycin. *Vet Immunol Immunopath 1985; 8:171-182*
67. FOUNTAIN MW, DEES C, SCHULTZ RD: Enhanced intracellular killing of *Staphylococcus aureus* by canine monocytes treated with liposomes containing amikacin, gentamycin, kanamycin, and tobramycin. *Current Microbiol 1981; 6:373-376*
68. BONVENTRE PF, GREGORIADIS G: Killing of intraphagocytic *Staphylococcus aureus* by dihydrostreptomycin entrapped within liposomes. *Antimicrob Agents Chemother 1978; 13:1049-1051*
69. STEVENSON M, BAILLIE AJ, RICHARDS RME: Enhanced activity of streptomycin and chloramphenicol against intracellular *Escherichia coli* in the J774 macrophage cell line mediated by liposome delivery. *Antimicrob Agents Chemother 1983; 24:742-749*
70. DESIDERIO JV, CAMPBELL SG: Intraphagocytic killing of *Salmonella typhimurium* by liposome encapsulated cephalothin. *J Infect Dis 1983; 148:563-570*
71. VLADIMIRSKY MA, LADIGINA GA: Antibacterial activity of liposome-entrapped streptomycin in mice infected with *Mycobacterium tuberculosis*. *Biomed Pharmacother 1982; 36:375-377*
72. KENDE M, ALVING CR, RILL WL, SWARTZ GM Jr, CANONICO PG: Enhanced efficacy of liposome encapsulated ribavirin against Rift Valley fever virus infection in mice. *Antimicrob Agents Chemother 1985; 27:903-907*
73. SIX HR, KRAMP WJ, KASEL JA: Effects of liposomes on serological responses following immunization with adenovirus purified type 5 subunit vaccines. In: *Liposomes and Immunobiology*. TOM BH, SIX HR (eds.) Elsevier-North Holland, New York, NY, 1980. pp. 119-131
74. SHEK PN, SABISTON BH: Immune response mediated by liposome-associated protein antigens. I. Potentiation of the plaque-forming cell response. *Immunology 1982; 45:349-356*
75. SHEK PN, YUNG BYK, STANACEV NZ: Physicochemical and immunological properties of albumin-associated dialkylether phosphatidylcholine liposomes. *Biochim Biophys Acta 1986; 855:33-40*
76. GERLIER D, SAKAI F, DORE JF: Induction of antibody response to liposome-associated Gross virus cell-surface antigen (GCSAa). *Br J Cancer 1980; 41:236-242*
77. FREISE J, FEUERHAKE A, MAGERSTEDT P, WILLIE E, MULLER R: The immunogenicity of hepatitis B surface antigen vaccine can be enhanced by encapsulation into liposomes. *Biol Cell 1983; 47:134*
78. YATVIN MB, LELKES PI: Clinical prospects for liposomes. *Medical Physics 1982; 9:149-175*
79. NISHIDA Y, KAMATANI N, MIYAMOTO T: Hypouricaemic effects of oral administration in chicken of polyethylene glycol-modified uricase entrapped in liposomes. *J Pharm Pharmacol 1984; 36:354-355*
80. OSBORNE MP, RICHARDSON VJ, PAYNE JH, MCREADY VR, RYMAN BE: Technetium-99m labelled liposome axillary lymphoscintigraphy in breast cancer. *Clin Oncol 1980; 6:289-290*
81. MORGAN JR, WILLIAMS LA, HOWARD CB: Technetium-labelled liposome imaging for deep-seated infection. *Br J Radiol 1985; 58:35-39*

(This paper is publication number 86-P-12 from the Defence and Civil Institute of Environmental Medicine.)